ORIGINAL ARTICLE

Effects of *Ammi visnaga* (Bisnaga) Extract on the Volume and Acidity of Stimulated Gastric Secretion in Fasting Rabbits

Muhammad Jan

Abstract

Objective: To determine the efficacy of extract of *Ammi visnaga* on volume and acidity of stimulated gastric secretion in rabbits and also its safety on liver and kidney function.

Study Design: Quasi experimental study.

Place and Duration of Study: Pharmacology Department, Saidu Medical College, Swat, in the years 2008-9.

Methodology: Thirty rabbits weighing 1 – 1.5 kg were divided into groups A, B and C each having 10 animals. After fasting for 48 hours, pylorus of animals of group A and B was ligated. Group A was administered Carbachol and group B was given extracts of *Ammi visnaga* followed by Carbachol after 15 minutes intraperitoneally. After 4 hours, stomach contents were measured for volume and then centrifuged and estimated for acidity. The extract was also administered to group C animals for 45 days to observe its effects on liver and kidney function.

Results: In group B, reduction in volume, free and total acidity of gastric juice was highly significant when the mean values were compared with group A. In group C, mean values of liver and kidney function test compared with pre-treated values, were found statistically non-significant.

Conclusion: *Ammi visnaga* extract can be used effectively and safely in the treatment of hyper acidity conditions and peptic ulcer after evaluation of its effects in human being.

Key Words: *Ammi visnaga. Gastric acid. Liver function. Kidney function.*

INTRODUCTION

Peptic ulcers are not found in achlorhydric patients and almost always occur in patients with Zollinger- Ellison (ZE) syndrome which is characterized by very high acid secretion.[1] It has been documented that 38 medicinal plants including fruits of *Ammi visnaga (Bisnaga, Ajwine)* in vernacular have a natural calcium channel blocker.[2] Khellin and Visnagin were identified from *Ammi visnaga* fruit having calcium channel blocking mode of action.[3-5] The extract from the fruit of *Ammi visnaga* is also used to treat asthma and angina pectoris[6] and also has nematocidal[7], insecticidal and repellant activity.[8] Visnagin has potential effects on kidney stone prevention,[9,10] a neuroprotective effect,[11] and anti-inflammatory effect.[12] Khellin and Visnagin prevent renal epithelial cell damage and play a potential role in the prevention of stone formation associated with hyperoxaluria.[13] It has bactericidal activity except against *Pseudomonas aerugenosa*.[14] Visnagin causes vasodilatation and reduces blood pressure by inhibiting calcium ion influx into the cell and has also anti-inflammatory effect in microglial cells.[15,16] Calcium channel blocking agents are commonly used in the treatment of hypertension, angina, myocardial infarction and supraventricular tachycardia,[17] as inhibitors of muscle contraction. Stomach motility and acid secretion have been shown to be dependent upon calcium ions influx making *Ammi visnaga* an option for the treatment of peptic ulceration.

Therefore, this study was conducted to evaluate the effects of extract from the fruits of *Ammi visnaga* on the volume and acidity of Carbachol induced gastric secretion and effects on liver and kidney function.

METHODOLOGY

Thirty rabbits of local breed were selected for this experimental study which was conducted at Pharmacology Department, Saidu Medical College, Swat, in the years 2008-9, after ethical approval from the Institute.

Healthy animals of either gender weighing 1-1.5 kg were used in the study. All the animals were kept fasting for 48 hours with free availability of water before they were subjected to experimental procedure. The animals were divided into 3 groups each containing 10 animals. Group A was Carbachol treated, group B was *Ammi visnaga* + Carbachol treated and group C was only *Ammi visnaga* treated for 45 days. Extract was prepared in Tradition Medicine Division in National Institute of Health (NIH), Islamabad. Fruit of *Ammi visnaga* was dried in shade. Twenty grams of finely grinded fruit of *Ammi visnaga* was dissolved in methanol for one time, put in Soxhelt extractor. Vapours were condensed and then dried to solid form. Dose was calculated in milligram on the basis of dry extract.

Department of Pharmacology, College of Medicine, University of Dammam, Dammam, Saudi Arabia.

Correspondence: Dr. Muhammad Jan, P. O. Box 2114, College of Medicine, University of Dammam, Dammam-31451, Saudi Arabia.
E-mail: drmuhammadjansmc@gmail.com

Received: April 19, 2011; Accepted: September 09, 2013.

The operative procedure was the one adopted by Vischer *et al*.[18] Animals were anaesthetized with ether in a dessicator, abdomen was opened and pylorus was ligated with silk suture. Then abdominal wall was closed with suture clamps and Carbachol 600 µg/kg body weight was administered intraperitoneally (I.P) to group A, *Ammi visnaga* 500 mg/kg body weight to group B followed by Carbachol 600 µg/kg body weight after 15 minutes to group B. The rabbits were deprived of water for 4 hours after the administration of drugs. Then the rabbits were sacrificed, thorax and abdomen were opened, esophagus was ligated and the stomach was removed quickly. The contents of the stomach were collected. The volume of gastric juice was measured. Then the contents were centrifuged, filtered and subjected to titration for estimation of free and total acidity by the method described by Varley.[19] Total acidity was the sum of free plus combined acidity. In case of group C, blood samples were taken for estimation of liver and kidney function before and after 45 days treatment with extract 500 mg/kg body weight twice daily intraperitoneally.

The data were entered into SPSS version 19. Means and SD (standard deviation) were calculated for all the quantitative variables, while frequencies and percentages were calculated for qualitative variables. Inferential test used to compare between the two groups was paired sample t-test for numerical variables and chi-square test for categorical variables. A p-value of < 0.05 was considered to be statistically significant.

RESULTS

The mean values of volume, free acidity and total acidity of gastric secretion in group A (Carbachol treated group) and group B (*Ammi visnaga* + Carbachol treated) were compared. There was reduction in the mean values of all the parameters mentioned above. These reductions were found to be statistically highly significant ($p < 0.001$, Table I). In group C, the mean values for kidney function (serum creatinine and blood urea) and liver function (serum bilirubin, SGPT and alkaline phosphatase) after administration of the extract of *Ammi visnaga* for 45 days were compared to the mean values of the above mentioned parameters from the samples taken before the administration of the extract (control). These changes were non-significant (Table II).

Table I: Effect of extract from *Ammi visnaga* (500 mg/kg body weight) on volume and acidity of Carbachol (600 µg/kg body weight) induced gastric secretion in rabbits.

Drug	Volume of gastric secretion (ml)	Acidity (meq/dl of gastric secretion)	
		Free	Total
Carbachol	28.7 ± 2.057 (10)	6.39 ± 1.29 (10)	7.64 ± 1.29 (10)
Ammi visnaga + Carbachol	13.8 ± 1.82 (10)	2.41 ± 0.68 (10)	3.57 ± 0.87 (10)
p-values (paired sample t-test)	< 0.001	< 0.001	< 0.001

All the values are given as mean ± SD

Table II: Effects of extract from *Ammi visnaga* on kidney function and liver function in rabbits after the administration of extract 500 mg/kg body weight intraperitoneally twice daily for 45 days.

Drug	Kidney function tests		Liver function tests		
	S. Creatinine (mg/dl)	Blood urea (mg/dl)	S.Bilirubin (mg/dl)	S.G.P.T (I.U/L)	Alk. phosphatase (I.U/L)
Carbachol (before injecting the extract)	1.24±0.08 (10)	36.2±2.93 (10)	0.17±0.08 (10)	36.1±2.33 (10)	82.6±2.17 (10)
Ammi visnaga	1.24±0.08 (10)	34.1±2.75 (10)	0.13±0.04 (10)	35.8±2.04 (10)	82.1±2.13 (10)
p-value (paired sample t-test)	0.919	0.112	0.168	0.627	0.685

All the values are given as mean ± SD

DISCUSSION

This study evaluated the effect of *Ammi visnaga* extract on the gastric acid production in a rodent models. Increased acid production from gastric mucosa is responsible for peptic ulceration in majority of the human patients. The chemical mediators acetylcholine and gastrin act through calcium ions influx and increase gastric acid secretion. Calcium also stimulates the release of gastrin. Cholinergic stimulation causes increased gastric acid secretion due to increase in the permeability of parietal cell membrane to extracellular calcium. Carbachol being a cholinomimetic agent increases free intracellular calcium ions that in turn activate protein kinase by phosphorylation and lead to increased production of HCl. It was, therefore, used to simulate the hyper acidity conditions in the animal model.

Induction of hypercalcaemia through intravenous administration of calcium is usually associated with increased gastric volume and acidity.[20,21] The acid stimulating ability of calcium is well known and there is extreme sensitivity to calcium in patients with ZE syndrome.[22,23] So calcium channel blockers may have a definite role in reducing gastric acid secretion.

In this study, it was observed that *Ammi visnaga* reduced the volume free acidity and total acidity in rabbits. All these reductions were statistically highly significant when compared with the mean values in Carbachol treated group. This is due to the calcium channel blocking activity of natural calcium channel blocker present in the extract.

This study results are consistent with other workers who concluded that calcium channel blocker Verapamil significantly reduces gastric acid secretion.[21,22] The extracts containing natural calcium channel blockers inhibit the calcium ion influx, which may be responsible for the observed reductions in volume and acidity of gastric secretion. Besides, calcium channel blockers inhibit lipoxygenase pathway during metabolism of arachidonic acid. So leukotrienes, the injurious substances are not formed and all the arachidonic acid is metabolized through the cyclo-oxygenase pathway. This will lead to the production of prostaglandin which

couples with GI protein. This inhibits adenyl cyclase. There is no proton pump activation and thus decrease HCl production.[23] Release of histamine from mast cells is critically dependent on external calcium ions, so by blocking calcium ions, can block histamine release which is a potent agent for HCl secretion.[24]

Calcium channel blockers are also used in controlling contraction of cardiovascular smooth muscles[25] and prevention of premature labor.[26] All these actions are due to the calcium channels blocking activity. Similarly, the anti-acid secretory action of the extract can be explained on the basis of documented calcium channel blocker present in the extract.

Therefore, it can be effectively used for hyper acidity conditions, peptic ulcer and the treatment of the above mentioned diseases. When the mean values of different parameters for liver and kidney function were compared before and after treatment, all the changes were non-significant showing that this extract is safe for these organs. There were no scientific or technical bias in this study. The major limitation of this study is that its results cannot be generalized to humans as the study was carried out on animals. Therefore, such study must be conducted first in human models with due precautions.

CONCLUSION

Ammi visnaga extract may be beneficially used in hyperacidic secretory conditions and peptic ulcer disease without any adverse effect on liver and kidneys. Further studies in this regard for evaluation of these effects are suggested in human subjects.

REFERENCES

1. Brage R, Cortijo J, Esplugues JK, Esplugues J, Bomnati E1, Edward CR, *et al*. Diseases of the stomach. In: Colledge NR, Walker BR, Raltson SH, editors. Davidson's principles and practice of medicine, London. *Churchill Livingstone* 1995; p. 425-34.
2. Azhar I, Aftab K, Usmanghani K. Naturally occurring calcium channel blockers. *Hamdard Medicus* 1995; **38**:5-16.
3. Rauwald HW, Brehm O, Odenthal KP. The evolvement of channel blocking mode of action in the pharmacology of *Ammi visnaga* fruits. *Planta Med* 1994; **60**:101-5.
4. Rauwald HW, Brehm O. Screening of some medicinal plants for their possible calcium antagonistic activity. *Planta Med* 1991; **7**:A59-A60.
5. el-Domiaty MM. Improved high-performance liquid chromatographic determination of khellin and visnagin in *Ammi visnaga* fruits and pharmaceutical formulations. *J Pharm Sci* 1992; **81**: 475-8.
6. Ichikawa K, Kinoshita T, Sankawa U. The screening of Chinese crude drugs for calcium antagonistic activity. Identification of active principles from the arial parts of the Pogostenum cablin and the fruits of *Prunus mume. Chem Pharm Bull* 1989; **37**: 345-8.
7. Park IK, Kim J, Lee SG, Shin SC. Nematicidal activity of plant essential oils and components from *Ajwain (Ammi)* All spice (Pimento divoca) and Litsea *(Litsea cubeba)* essential oil against pine wood nematode (Bursaphelenchus xylophilus). *J Nematol* 2007; **39**:275-9.
8. Pandy SK, Upadhyas S, Ttriphathi AK. Insecticidal and repellant activities of Thymol from the essential oil of *Trachyspermum ammi* (Linn) sprague against anopheles stephensi. *Parasitol Res* 2009; **105**:507-12. Epub 2009 Apr 3.
9. Haug KG, Weber B, Hochhaus G, Butterweck V. Non-linear pharmacokinetics of visnagin in rats after intravenous bolus administration. *Eur J Pharm Sci* 2012; **45**:79-89.
10. Meiouet F, El Kabbaj S, Daudon M. *In vitro* study of the litholytic effects of herbal extracts on cystine urinary calculi. *Prog Urol* 2011; **21**:40-7. Epub 2010 Jul 6.
11. Kwon MS, Lee JK, Park SH, Sim YB, Jung JS, Won MH, *et al*. Neuroprotective effect of visnagin on kainic acid induced neuronal cell death in the mice hippocampus. *Korean J Physiol Pharmacol* 2010; **14**:257-63. Epub 2010 Oct 31.
12. Lee JK, Jung JS, Park SH, Park SH, Sim YB, Kim SM, *et al*. Anti-inflammatory effect of visnagin in lipopolysaccharide stimulated BV-2 microglial cells. *Arch Pharm Res* 2010; **33**: 1843-50. Epub 2010 Nov 30.
13. Vanachayangkul P, Byer K, Khan S, Butterweck V. An aqueous extract of *Ammi visnaga* fruits and its constituent's khellin and visnagin prevent cell damage caused by oxalate in renal epithelial cells. *Phytomedicine* 2010; **17**:653-8. Epub 2009 Dec 29.
14. Mayaud L, Carricajo A, Zhiri A, Aubert G. Comparision of bacteriostatic and bactericidal activity of 13 essential oils against strains with various sensitivity to antibiotics. *Lett Appl Microbiol* 2008; **47**:167-73.
15. Lee JK, Jung JS, Park H, Sim YB, Kim SM, Ha TS, *et al*. Anti-inflammatory effect of visnagin in lipopolisaccharide stimulated BV-2 microglial cells. *Arch Pharma Res* 2010; **33**:1843-50. Epub 2010 Nov 30.
16. Kwon SM, Lee JK, Park SH, Sim YB, Jung JS, Won MH, *et al*. Neuro-protective effect of visnagin on kainic acid - induced neuronal cell death in the mice hypocampus. *Korean J Physiol Pharmacol* 2010; **14**:257-63.
17. Fleckenstein A. History of calcium antagonists. *Circ Res* 1983; **52**:3-16.
18. Vischer FE, Seay PH, Tazelaar AP, Veldkamp JW, Brook MJ. Pharmacology of famine bromide. *J Pharacol Expt Ther* 1954; **110**:188-204.
19. Varley H. Practical clinical biochemistry. London: *Willium Meinmann* 1996; p. 249-77.
20. Barreras RF. Calcium and gastric secretion. *Gastroenterology* 1973; **64**:1168- 84.
21. Anderson JR. Diseases of the stomach. In: Levison D, Reid R, Burt AD, Harrison DJ, Fleming S, editors. Muir's text book of pathology. London: English Language Book Society/Edward. *Arnold;* 1985; p. 19-26.
22. Basso N, Materia A, Folini A, Jafe BM. Prostaglandin generation in the gastric mucosa of rats with stress ulcer. *Surgery* 1983; **94**:104-8.
23. Passaro EJ, Basso N, Walsh JH. Calcium challenge in Zollinger-Ellision syndrome. *Surgery* 1972; **72**:60-7.

24. Kirkegaard P, Christiansen J, Petersen B, Skov Olsen P. Calcium and stimulus-secretion coupling in gastric fundic mucosa: effect of inhibition of calcium transport by Verapamil on gastric acid secretion in the isolated guinea pig fundic mucosa and in healthy subjects. *Scand J Gastroenterol* 1982; **17**:533-8.

25. Flekanstein A. Specific pharmacology of calcium in peri-cardium, cardiac pacemaker and vascular smooth muscles. *Ann Rev Pharmacol Toxicol* 1977; **17**:149-66.

26. Latif A, Aamir K, Ali M, Siddiqi N. Deactivation of ripened uterine tissue by calcium channel blocker Verapamil. *Pak J Pharmacol* 2002; **19**:13-7.